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Biogenic amines Formation in Fish Sauce Prepared from Fresh and Temperature-Abused Indian Anchovy (*Stolephorus indicus*)

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Abstract

The formation of biogenic amines in fish sauce made from fresh and temperature-abused (left at 35 °C for 8 and 16 h) Indian anchovy (*Stolephorus indicus*) was investigated. Histamine, cadaverine, putrescine, and tyramine were predominant biogenic amines found in anchovy left at 35 °C for 16 h and its fish sauce product. Changes of biogenic amines were subtle during the course of fermentation at room temperature (RT) and at 40 °C, suggesting that the main source of biogenic amines was associated with raw material, rather than with fermentation process. Soluble peptide and total nitrogen of fish sauce prepared from temperature-abused anchovy were higher at the initial stage of fermentation at RT and 40 °C and became comparable to those prepared from fresh anchovy at the end of fermentation. Total free amino acid contents of samples fermented at RT for 52 wk (7,208.3-8,473.6 mg/100 mL) were higher than those fermented at 40 °C for 13 wk (4,560.9-5,730.9 mg/100 mL). Fish sauce prepared from temperature-abused anchovy contained less free histidine and arginine. Good quality of fish sauce was obtained using fresh anchovy fermented at RT. Besides total nitrogen content, biogenic amines should be considered as quality indicators of fish sauce.

Key words: Biogenic amines, fish sauce, anchovy, *Stolephorus indicus*

Introduction

Fish sauce is clear amber liquid with a unique aroma and flavor and is rich in amino acids (Saisithi 1994). It is widely used as a condiment and seasoning in most countries of Southeast Asia and gradually gained acceptance worldwide. Fish sauce is basically a protein hydrolysate resulted from a natural fermentation of fish and salt at the ratio of 3:1. The mixture is kept in tanks at ambient temperature for 12-18 mo (Saisithi 1994). Protein hydrolysis is induced by endogenous proteinases in fish muscle and digestive tract as well as proteinases produced by halophilic bacteria (Gildberg and Thongthai 2001; Saisithi 1994). Degradation of fish protein to free amino acids is primarily responsible for delicious taste of fish sauce (Chayovan and others 1983). Various volatile compounds, including acids, carbonyls, nitrogen-containing compounds, and sulfur-containing compounds, are formed during fermentation and thought to be responsible for distinct aroma of fish sauce (Fukami and others 2002; Peralta and others 1996; Shimoda and others 1996).

The main species for fish sauce production in Southeast Asia is Indian anchovy (*Stolephorus spp.)*. Rodtong and others (2004) reported that histamine content of Indian anchovy sharply increased to 93.3 mg/100 g after storage at 35 °C for 16 h. Anchovy is normally caught and kept on board without ice up to 8 h before landing. Fish is typically transported to a factory in an open container without a proper cooling system. Therefore, fish is practically subjected to temperature abuse before being processed. Commercial fish sauce made from anchovy was found to contain histamine ranging from 10 to 100 mg/100 mL (Brillantes and Samosorn 2002). The maximum allowable histamine in fish sauce imposed by the Canadian Food Inspection Agency (CFIA) is 20 mg/100 g (CFIA

2003). Therefore, some commercial fish sauce products contain histamine exceeding the Canadian standard. Few studies have been conducted to identify the cause of histamine formation in fish sauce. Brillantes and others (2002) reported that high level of histamine content in fish sauce was contributed from raw material and during fermentation. However, Sanceda and others (1999) suggested that histamine content decreased during fermentation due to the action of histamine-decomposing bacteria.

Besides histamine, other biogenic amines, including cadaverine, tyramine, putrescine spermine, and spermidine are also accumulated when fish undergo spoilage. These compounds are resulted from bacterial decarboxylation of various amino acids (Silla Santos 1996). The toxicity of histamine appears to be enhanced by cadaverine and putrescine because they inhibit histamine-detoxifying enzymes: diamine oxidase and histamine N-methyltransferase (Stratton and others 1991). Tyramine was accumulated to levels in cheese that were toxicologically significant (ten Brink and others 1990). Formation of biogenic amines were found in various fish species, including anchovy (*Engraulis encrasicholus*), albacore tuna (*Thunnus alalunga*), Bigeye tuna (*Thunnus obesus*), and Skipjack tuna (*Katsuwonus pelamis*) (Ben-Gigirey and others 1998; Kim and others 1998; Rossi and others 200; Veciana-Nogués and others 1996). However, formation of biogenic amines in Indian anchovy and anchovy fish sauce has not yet been reported.

Lopetcharat and Park (2002) reported that endogenous proteinase(s) played an important role in protein hydrolysis during high salt (25%) fermentation of whole Pacific whiting (*Merluccius productus*) and its surimi byproducts. The highest proteolytic activity was found at 50-65 °C. Yield and total nitrogen of samples fermented at 50 °C were higher than those at 35 °C. Therefore, increasing fermentation temperature could result in high degree of hydrolysis, which could in turn lead to acceleration of fermentation process. However, the effect of high temperature fermentation on the extent of hydrolysis and biogenic amine formation has not yet been evaluated.

The presence of biogenic amines in fish sauce does not pose any health threat compared to other fishery products because average uptake of fish sauce is relatively small, about 20 mL/person/d (Anonymous 2000). However, it implies poor hygienic qualities of raw material and/or manufacturing processes. The objectives of our study were to investigate changes of biogenic amines during fish sauce fermentation with respect to freshness quality of Indian anchovy. The extent of protein hydrolysis as affected by varied degree of freshness qualities and fermentation temperature was also elucidated. In addition, potential indicators for fish sauce quality were sought.

Materials and Methods

Samples and sample treatment

Indian anchovy (*Stolephorus indicus*) were caught off the Gulf of Thailand at Chonburi province. Samples were kept in ice on board and transported to the Suranaree University laboratory. Upon arrival, samples were allocated into 3 portions. The first portion was immediately used for fish sauce fermentation and designated as fresh fish (F). The second and third portion of fish was left at 35 °C for 8 (8h) and 16 h (16h), respectively, to induce a varied degree of decomposition. Since the average ambient temperature in Thailand is around 28-32 °C and anchovy is typically exposed to sunlight during transportation, relatively high temperature (35 °C) was chosen to induce decomposition in this study. F, 8h, and 16h samples were analyzed for biogenic amines, soluble oligopeptides, trimethylamine (TMA), and total volatile base nitrogen (TVB-N).

Fish sauce samples were prepared using F, 8h, and 16h as a raw material. Each fish sample was mixed with solar salt at a ratio 7:3. Each mixture (5 kg) was then packed in a 6-L glass jar (diameter of 17 cm and height of 27 cm), occupied about 90% of total jar volume. Each jar was covered with a glass plate. Fermentation was carried out at ambient (28-32 °C) for 52 wk and in a 40 °C incubator for 13 wk (Hotpack an SP Industries Co., Philadelphia, PA). Fermentation at 40 °C was selected in this study because our preliminary studies indicated that fermentation at higher temperatures (50-65 °C) resulted in a cooked flavor, which was objectionable. Samples (30-40 g) were taken at each time interval and centrifuged at 8000 rpm (PK 121R, ALC International Srl, Italy) at 4 °C for 10 min. The supernatant was collected and analyzed for biogenic amines, total nitrogen (TN), and α-amino content. After fermentation was completed, free amino acid profiles of each sample were analyzed.

Commercial fish sauce samples were obtained from the local supermarket at Nakhon Ratchasima and some were obtained from the fish sauce factory at Rayong. These are typical brands consumed domestically. These samples were made from Indian anchovy and fermented at ambient temperature. Biogenic amines, total nitrogen, and soluble peptides of these samples were analyzed.

Biogenic amine analyses

Determination of histamine, cadaverine, tyramine, putrescine, spermidine, and spermine were carried out by high-performance liquid chromatography (HPLC) by the method of with slight modifications. Histamine dihydrochloride, cadaverine dihydrochloride, tyramine hydrochloride, putrescine dihydrochloride, spermidine trihydrochloride, and spermine diphosphate (Sigma Chemical Co., St. Louis, MO, USA) were separately prepared in deionized water at a concentration of 1000 mg/L. Amine working solutions at 50 mg/L were prepared by diluting the stock solution with deionized water. Internal standard solution (1000 mg/L) of 1,7-diaminoheptane (Sigma Chemical Co., St. Louis, MO, USA) was also prepared.

Biogenic amines of fish tissue were extracted by adding 15 mL of 0.4 M perchloric acid to 5 g of homogenized fish sample, followed by the addition of 125 μL of internal standard solution as described by Eerola and others (1993). Biogenic amines of fish sauce samples were determined directly without extraction. Fish sauce samples were diluted with 0.4 M perchloric acid at either 2 or 200 times and mixed with internal standard to contain a final concentration of 1 mg/L. Recoveries of individual biogenic amines were determined by adding working standard solutions at 10-100 mg/L to fish and fish sauce samples before extraction. Subsequently, the internal standard was added and total volume was adjusted as described above.

One milliliter of extract or diluted extract was mixed with 200 μL of 2 N sodium hydroxide and 300 μL of saturated sodium bicarbonate. Two milliliters of dansyl chloride solution (10 mg/mL) prepared in acetone were added to the mixture, and then were incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 300 μL of 30% ammonia. After 30 min at room temperature, the extracts were adjusted to 5 mL with acetonitrile. The solution was filtered through a 0.45 μm regenerated cellulose membrane filter (Agilent Technologies, Inc., Germany).

A HPLC unit (HP 1100, Agilent Technologies, Inc., Palo Alto, Calif. USA) equipped with a photodiode array detector and HP ChemStation software (Rev.A.09.03) was employed. A Hypersil BDS C_{18}, (100×4 mm I.D., 3μm, 100 Å) reverse phase column fitted with a Hypersil BDS C_{18} (4×4 mm I.D., 5μm, 100 Å) guard column was used, with 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B) as a mobile phase at the flow rate of 0.2 mL/min. Isocratic elution was initiated with 50% solvent B for 5 min, subsequently the gradient elution was started and ended at 90% solvent B in 25 min. The column was equilibrated with 50% solvent A and B for 23 min before the next injection. The column was kept at 40 °C in a heated column compartment. The sample volume injected was 10 μl and the dansylated amines were detected at 254 nm with 550 nm as reference.

Trimethylamine (TMA) and total volatile base-nitrogen (TVB-N)

Trimethylamine (TMA) of raw materials were determined by the Modified Dyer Picrate method (AOAC, 1995). Whole fish (20 g) was homogenized in 80 mL cold 7.5% (w/v) trichloroacetic acid. The homogenate was centrifuged at 8000 rpm (PK 121R, ALC International Srl) at 4 °C for 10 min. The supernatant was further extracted in toluene and reacted with 1% picric acid. Absorbance was measured at 410 nm using trimethylamine as a standard. TMA was expressed as mg-TMA/100g.

Total volatile base-nitrogen (TVB-N) of raw materials was determined by the steam distillation as described by Botta et al. (1984). Ten grams of homogenized anchovy was added 2 g MgO and 40 mL distilled water. Steam distillation was performed using Kjeldahl distillation unit (Vapordest 30, Gerhardt, Germany) for 5 min.

The distillate was titrated with 0.1 N HCl and TVB-N was calculated and expressed as mg-N/100g sample.

α-Amino content

Soluble peptides of raw material and fish sauce were measured as α-amino content by the trinitrobenzenesulfonic acid (TNBS) method (Adler-Nissen 1979). Liquid obtained after centrifugation at each time interval was diluted with 1% sodium dodecyl sulfate (SDS) about 50-200 times, depending on the sample. One hundred microliters of diluted sample was mixed with 1 mL of 0.2125 M phosphate buffer (pH 8.2). One milliliter of 0.05% TNBS solution was added and thoroughly mixed, and then placed in a water bath at 50°C for 1 h. The reaction was terminated by adding 2 mL of 0.1 N HCl. Absorbance was measured at 420 nm using leucine as a standard. α-Amino content was expressed as mM of leucine.

Total nitrogen content

Total nitrogen of liquid centrifuged at each time interval was measured using the micro-Kjeldahl method (AOAC, 1995). Digestion was performed using a Kjeldhtherm 30 (Gerhardt, Germany). Distillation was achieved using a Vapordest 30 distillation unit (Gerhardt, Germany). Total nitrogen was expressed as g-N/100mL.

Free amino acid profiles

Fish sauce samples fermented for 13 and 52 wk at 40 °C and room temperature, respectively, were used for free amino acid analysis as described by Tungkawachara and others (2003). A mixture of 5 mL of fish sauce and 250 mg of 5'-sulfosalicylic acid (SSA) was allowed to stand for 1 h at cold room. The mixture was centrifuged at 10000 × g for 15 min to remove precipitated protein. The supernatant was made up to 50 mL with a lithium citrate buffer, pH 2.2 (Biochrom Ltd, Cambridge, England), and filter through a 0.2 μm membrane filter. Filtered sample (40 μl) was injected into an amino acid analyzer (Biochrom 20 plus, Biochrom Ltd, Cambridge, England). The standard amino acid for physiological fluid was analyzed in the same condition to identify retention time. The amount of free amino acid was expressed as mg of amino acid per 100 mL of fish sauce.

Statistical analyses

All chemical analysis was carried out at least in duplicate. One-way analysis of variance (ANOVA) was performed to determine the differences between periods of fermentation. Two-way ANOVA was used to determine significant differences between treatments (raw material × fermentation temperature). Duncan's multiple range test (DMRT) was used to determine differences between mean at $p < 0.05$ (SAS Institute, Inc., NC, USA).

Results and Discussion

Biogenic amines of raw material and during fish sauce fermentation

The recovery of all biogenic amines ranged from 96.1-117.5%. Tryptamine appeared to coelute with other amino acids and/or peptides, resulting in higher recovery of 117.5%. Thus, tryptamine content reported in our study was likely to be overestimated. Fresh (F) anchovy contained relatively low biogenic amines (Table 1). Decomposition progressed with prolonged storage time at 35 °C as evident by an increase in TMA, TVB-N, and biogenic amines. Based on visual examination, the 8h sample was considered as moderately fresh quality, while the 16h sample was in the decomposed state exhibiting severe tissue softening, excessive slime, and putrid odor. High level of histamine (200.7 mg/100g), cadaverine (86.3 mg/100g), tyramine (27.3 mg/100g), and putrescine (26.0 mg/100g) were found in the 16h sample. Putrescine and cadaverine have been reported as potentiators for histamine toxicity (Silla Santos, 1996). Therefore, anchovy exposed to 35 °C for 16 h was not suitable for human consumption. Putrescine and cadaverine were detected in decomposed gilt-head sea bream (*Sparus aurata*) (Koutsoumanis and others 1999). Histamine, cadaverine, tyramine, and putrescine were important amines formed during spoilage of anchovy (*Engraulis encrasicholus*) at 8 and 22 °C (Veciana-Nogués and others 1996). High level of cadaverine was also detected in Bigeye tuna (*Thunnus obesus*) and Skipjack (*Katsuwonus pelamis*) stored at room temperature (Rossi and others 2002). Our results indicated that cadaverine, tyramine, and putrescine were also accumulated along with histamine as Indian anchovy underwent decomposition.

When F sample was used as a raw material for fish sauce fermentation at room temperature (F/RT), low level of biogenic amine was found throughout 52 wk period (Figure 1a). Histamine was the predominant amine and gradually increased from 0.3 mg/100 mL to 0.9 mg/100 mL in 52 wk. At 40 °C fermentation, fish sauce was obtained after 13 wk based on total nitrogen content (Figure 3b) and amber color as well as fish sauce aroma. Changes of biogenic amines in F/40C (fresh anchovy fermented at 40 °C) were similar to those of F/RT (Figures 1a,2a). It should be noted that histamine increased to a greater extent when fermentation was carried out at 40 °C. A negligible level of histamine (1.6 mg/100 mL) was also found in the finished product of F/40C. Histamine-forming bacteria isolated from Indian anchovy, namely *Morganella morganii*, *Proteus vulgaris*, and *Enterobacter aerogenes*, did not grow and produce histamine at 20-25% NaCl (Rodtong and others 2004). Since salt contents of all samples were approximately 25-26%, bacterial flora of histamine-formers were unlikely to cause an increase in histamine during fermentation. Histamine-forming halophilic bacteria isolated from salted anchovy (*Engraulis encrasicholus*) and other fermented fish products included *Staphylococcus epidermidis*, *S. captitis*, and *Tetragenococcus muriaticus* (Hernández-Herrero and others 1999; Kimura and others 2001). However, these bacteria were not isolated from our samples (data not shown). A small increase in histamine formation of F/RT and F/40C might have occurred as a consequence of the activity of histidine decarboxylases secreted by histamine-forming bacteria before the fermentation, rather than from halophilic bacteria during fermentation.

Cadaverine and histamine were major amines found in fish sauce made from 8h samples fermented at both RT and 40 °C (Figures 1b, 2b). Although histamine in samples prepared from F and 8h appeared to increase during fermentation at both temperatures, histamine contents of finished products were far below an allowable limit of 20 mg/100 mL (CFIA 2003). It was concluded that an increase in biogenic amines during fermentation of fish sauce made from fresh and moderately fresh anchovy was insignificant.

Histamine, cadaverine, putrescine, and tyramine were found at high level in fish sauce made from the temperature-abused fish (16h) fermented at both temperatures (Figures 1c, 2c). Changes of these amines during fermentation were subtle at both fermentation temperatures. There was no difference in biogenic amines content between 16h/RT and 16h/40C ($p > 0.05$). Sanceda and others (1999) also reported subtle changes of histamine during 50 d of fish sauce fermentation using 20-30% salt. In our study, spermine, spermidine, and tryptamine remained <10 mg/100 mL throughout fermentation period at both temperatures (Figures 1c, 2c). Kirschbaum and others (2000) also reported high level of histamine (72.1-75.7 mg/100 mL), cadaverine (10.8-28.5 mg/100 mL), tyramine (33.7-73.9 mg/100 mL), and putrescine (10.8-19.7 mg/100 mL) in anchovy fish sauce. These four amines were also detected at relatively high content in salted and fermented anchovy products from Korea (Mah and others 2002). Therefore, these four biogenic amines were also prevalent in various fermented anchovy products.

Histamine content of commercial products varied from 20.97 to 78.30 mg/100 mL (Table 2), which exceeded the CFIA limit. In addition, majority of samples contained histamine over 50 mg/100 mL, the maximum limit for fish sauce in Thailand (FIQD 2000). Cadaverine, putrescine, and tyramine were also found in relatively high level in concomitant with histamine. Based on our results, these 4 biogenic amines were also predominant in fish sauce prepared from decomposed fish. Due to subtle changes of these biogenic amines during fermentation, their main source appeared to derive from raw material. It could be speculated that some commercial products could be made from temperature-abused raw material. Therefore, histamine, cadaverine, putrescine, and tyramine could be potential indicators of fish sauce quality. High level of these amines would indicate poor hygienic quality of raw material.

Changes of α-amino content

Proteolysis results in the formation of α-amino groups of either oligopeptides and amino acids, which can react with TNBS to form sulfite complex of the trinitrophenylated amino groups (Fields, 1971). Therefore, an increase in α-amino content indicates the extent of protein hydrolysis. α-Amino contents of the samples prepared from 16h were higher than those prepared from 8h and F, respectively (Figure 3a,b). This was due to a greater extent of proteolysis induced by endogenous and microbial proteinases in 16h sample (Table 2). Soluble oligopeptide increased as incubation time at 35 °C was prolonged (Table 2). As fermentation progressed, protein hydrolysis gradually increased by the action of proteinases. Orejana and Liston (1982) suggested that trypsin-like proteinases from digestive tract of anchovy (*Stolephorus* spp.) were responsible for protein hydrolysis during the first 20 wk of fermentation. In addition, protein hydrolysis of fish sauce was contributed from aminopeptidases associated with internal organ of fish (Vo-Van and others 1984). Besides the involvement of endogenous proteinases, extracellular proteinases produced by halophilic bacteria were also suggested to participate in protein hydrolysis during fish sauce fermentation. *Halobacillus thailandensis* isolated from Thai fish sauce produced extracellular proteinases that catalyzed hydrolysis of gelatin at NaCl concentration up to 30% (Chaiyanan and others 1999).

An increase of soluble peptides extensively took place during the initial stage of fermentation, especially within the first 13 wk of fermentation at RT (Figure 3a). Protein hydrolysis appeared to be minimal thereafter as evident by a slight increase in α-amino content from 736-822 mM at 13 wk to 860-878 mM at 52 wk (Figure 3a). This could be due to decreased proteolytic activity in the presence of high salt content (25%). Aminopeptidase activity in sardine fish sauce gradually decreased and reached the minimum within 6 mo of fermentation (Vo-Van and others 1984). A number of halophilic bacteria also declined as fermentation progressed (Gildberg and Thongthai 2001; Saisithi 1994). It should be noted that development of color, aroma, and flavor extensively occurred during 13-52 wk. Although liquid collected at 13 wk contained relatively high α-amino content (736-822 mM), it was turbid and exhibited fishy odor. Therefore, reactions involved in color and aroma development during 13-52 wk were important and contributed to unique characteristics of fish sauce. Our study also demonstrated that using spoiled fish (16h) did not accelerate fermentation, as being understood among some local manufacturers. Higher α-amino content of fish sauce made from 16h was only observed during 33 wk (Figure 3a). There were no differences in α-amino content among 3 samples (F, 8h, 16h) thereafter ($p > 0.05$). Since the extent of proteolysis is limited by concentration of protein substrate, the attainable degree hydrolysis of 3 samples, which contained essentially the same amount of protein content, were comparable at the end of fermentation.

Soluble peptides of all samples fermented at 40 °C during the first 10 wk were higher than those at RT (Figure 3a,b). Higher temperature could increase proteolytic activity of proteinases, subsequently increased degree of hydrolysis. Orejana and Liston

(1982) reported that trypsin-like activity recovered from round scad (*Decapterus* spp.) fish sauce increased with temperature and reached the optimum at 63 °C. Fermentation at 40 °C for 13 wk resulted in fish sauce containing about 700-800 mM α-amino contents, regardless of freshness quality of raw material. The samples exhibited amber color and typical flavor as well as aroma of fish sauce. Fermentation at 40 °C could be used as a means to accelerate fish sauce fermentation process.

Changes of total nitrogen

The release of water-soluble proteins from cells by osmotic pressure and degradation of muscle proteins to peptides and amino acids by proteolytic enzymes resulted in an increased total nitrogen content (Saisithi 1994). Therefore, changes of total nitrogen content of all samples were resemble to changes of α-amino contents (Figures 4a,b). Total nitrogen increased to reach the plateau of 2.1-2.3 g-N/100 mL in 25 wk, and remained relatively constant until 52 wk at room temperature (Figure 4a). Total nitrogen content of samples fermented at 40 °C increased at a faster rate compared to those fermented at room temperature and reached 2 g-N/100mL within 7 wk (Figure 4b).

Total nitrogen content is normally used as the only indicator to determine fish sauce quality and price. The product with total nitrogen content > 2 g-N/100 mL is classified as Grade I according to the Thailand Industrial Standard Institute (TISI) (Brillantes and others 2002; Saisithi, 1994). All samples fermented at RT and 40 °C contained total nitrogen content ranged from 2.1 to 2.5 g-N/100 mL, which were considered as Grade I fish sauce. Based on such standard, fish sauce prepared from 16h containing high levels of biogenic amines would have the same product quality as the low biogenic amine fish sauce made from F. Some commercial products classified as premium grade (TN > 2 g-N/100 mL) also contained high level of biogenic amines (Table 3). It was clear that total nitrogen content did not reflect quality of raw material used. Therefore, total nitrogen should not be the sole indicator for fish sauce grading. Biogenic amines content would provide useful information regarding the freshness quality of raw material used in fish sauce production.

Free amino acids of finished products

Free amino acids of fish sauce fermented at room temperature for 52 wk were higher than those at 40 °C for 13 wk regardless of raw material used (Figures 5a,b). Based on 18 free amino acids analyzed, total amino acid contents of samples fermented at room temperature and 40 °C were 7,208.3-8,473.6 mg/100 mL and 4,560.9-5,730.9 mg/100 mL, respectively. Glutamic was a predominant amino acid found in all fish sauce samples fermented at RT and 40 °C with concentrations of 1,144.6-1,176.5 mg/100 mL and 571.4-733.7 mg/100mL, respectively. Free glutamic acid was also a major amino acids found in fish sauce made from Pacific whiting (Tungkawachara and others 2003) and those produced in Asian countries (Park and others 2001). Amino acids contributing to sweet taste, namely glycine, alanine, threonine, proline, and serine, were also contained at greater content in samples fermented at RT (Figure 5a). Freshness quality of raw material did not affect glutamic content ($p>0.05$), but fermentation at 40 °C resulted in lower glutamic content ($p<0.05$). Since glutamic acid provides a good taste known as umami (Mizutani and others 1992), samples fermented at 40 °C could be perceived as less tasty. A shorter fermentation time at 40 °C limited the extent of protein hydrolysis, resulting in lower total free amino acids and glutamic acid content. It is noteworthy that free amino acid contents of samples fermented at RT were almost 2 times greater than those fermented at 40 °C, while their α-amino contents were comparable (Figure 3a). It could be speculated that samples fermented at 40 °C contained more oligopeptides, which also reacted with TNBS, resulting in comparable α-amino contents to those fermented at RT. Hydrolysis of oligopeptides to free amino acids might be necessary to improve sensory characteristics of samples fermented at 40 °C. Application of exopeptidases and/or prolonged fermentation time could increase free amino acids of samples fermented at 40 °C.

Histidine and arginine in fish sauce prepared from F were higher than those made from 8h and 16h at both fermentation temperatures ($p<0.05$) (Figure. 5a-b). Histidine of 16h was likely to be converted to histamine via the decarboxylation pathway (Silla Santos 1996). For this reason, 16h fish would contain lower amount of histidine compared to F fish, resulting in a lower histidine content in 16h fish sauce. Arginine is also metabolized to ornithine, which is further decarboxylated to putrescine by bacteria (Silla Santos 1996). In addition, arginine is converted to ornithine and citrulline via the Krebs-Hanseleit urea cycle (Mathews and van Holde 1990). Therefore, fish sauce made from temperature-abused fish contained higher level of putrescine (Figures. 1c,2c), ornithine and citrulline (Table 4) and lower level of arginine.

Taurine (2-aminoethansulfonic acid) contents were comparable among samples fermented at the same temperature (Table 3). It has been demonstrated that low level of taurine are associated with several pathological lesions, including cardiomyopathy, retinal degeneration, and growth retardation in various species (Huxtable 1992). Our study showed that fermented fish sauce is one of good sources of taurine, ranging from 116.3 to 156.6 mg/100 mL. Ammonium chloride indicating ammonia content of fish sauce samples, increased as decomposed raw material was used (Table 3). Samples fermented at RT contained higher ammonia than those at 40 °C ($p<0.05$). Decomposed anchovy contained higher volatile base compounds including ammonia, which were resulted from the action of spoilage bacteria. Fish sauce samples made from 16h, therefore, contained higher ammonia content than those made from F and 8h. McIver and others (1982) reported that ammonia and trimethylamine were predominant basic nitrogenous compounds contributing to an ammoniacal note. Therefore, raw material with low freshness quality would result in high level of biogenic amines and ammoniacal odor.

Conclusions

Histamine, cadaverine, putrescine, and tyramine were major biogenic amines found in fish sauce prepared from temperature-abused anchovy (16h). Changes of biogenic amines during fish sauce fermentation were subtle, suggesting that raw material, rather than a fermentation process, was a major source of biogenic amines. Utilization of temperature-abused raw material did not accelerate fermentation process. Biogenic amines could be used as quality indicators in conjunction with total nitrogen content.

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Table 1 Biogenic amines and other chemical indicators of Indian anchovy stored at 35 °C for various time.

Chemical indicator (mg/100g)	F	8h	16h
Tryptamine	N.D.	1.63±0.03	14.73±0.13
Putrescine	N.D.	1.38±0.04	25.99±0.12
Cadaverine	1.55±0.04	3.81±0.01	86.34±0.83
Histamine	1.40±0.002	3.28±0.001	200.70±0.94
Tyramine	4.69±0.13	5.44±0.08	27.30±0.99
Spermidine	4.93±0.09	7.14±0.03	5.52±0.14
Spermine	0.62±0.04	0.78±0.001	2.71±0.03
TMA	3.84±0.08	8.83±0.23	21.94±0.22
TVB-N[1]	30.52±0.31	46.47±2.05	90.12±0.92
Soluble oligopeptide[2]	79.02±3.21	118.06±2.42	143.26±2.17

[1] mg-N/100g
[2] mmole/100g
F = samples kept in ice after catch, 8h = samples incubated at 35 °C for 8 h, 16h = samples stored at 35 °C for 16 h.
N.D. = not detected

Table 2 Biogenic amines, total nitrogen, and α-amino content of commercial fish sauce samples[1].

Sample	Biogenic amine (mg/100 ml)							Total Nitrogen (g-N/100 ml)	Soluble peptides[2] (mM)
	Tryptamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine		
C1	3.05±2.20	30.82±1.75	68.55±4.09	57.47±4.77	11.73±1.27	0.99±0.07	0.37±0.007	2.72±0.05	1062.69±20.93
C2	1.24±0.01	4.21±0.51	8.66±0.91	20.97±2.72	0.44±0.07	0.28±0.06	0.06±0.03	2.04±0.05	828.48±17.24
C3	1.99±0.24	3.41±0.20	8.66±0.81	14.14±1.22	0.37±0.006	0.26±0.002	0.05±0.008	2.08±0.06	914.68±20.93
C4	4.55±0.12	47.22±1.91	75.57±3.12	78.30±4.27	23.19±0.66	1.73±0.10	0.77±0.002	1.92±0.02	750.13±4.93
C5	5.80±0.13	36.74±1.69	60.35±2.83	60.81±2.74	23.77±1.00	1.87±0.05	0.82±0.03	1.55±0.005	616.04±2.46
C6	3.29±0.48	15.52±1.12	29.07±2.15	31.70±2.45	10.34±0.59	4.95±0.08	1.06±0.19	1.04±0.005	400.99±8.62

[1] Means±standard deviation

Table 3 Amino acids and nitrogenous compound detected in fish sauce samples (mg/100 mL)

Compounds	RT fermentation			40 °C fermentation		
	F	8h	16h	F	8h	16h
Taurine	156.59	148.87	150.70	133.42	116.32	147.35
L-Citrulline	44.93	125.32	766.40	34.71	50.49	572.04
L-Cystine	105.21	67.26	66.70	78.28	63.32	74.39
L-Ornithine	12.38	21.87	38.50	6.76	9.99	38.02
Homocystine	5.86	5.96	8.80	6.93	5.38	7.68
Ethanolamine	19.99	19.92	15.90	21.10	17.11	20.68
Ammonium chloride	314.50	339.82	594.30	226.37	217.34	439.58

Figure legends

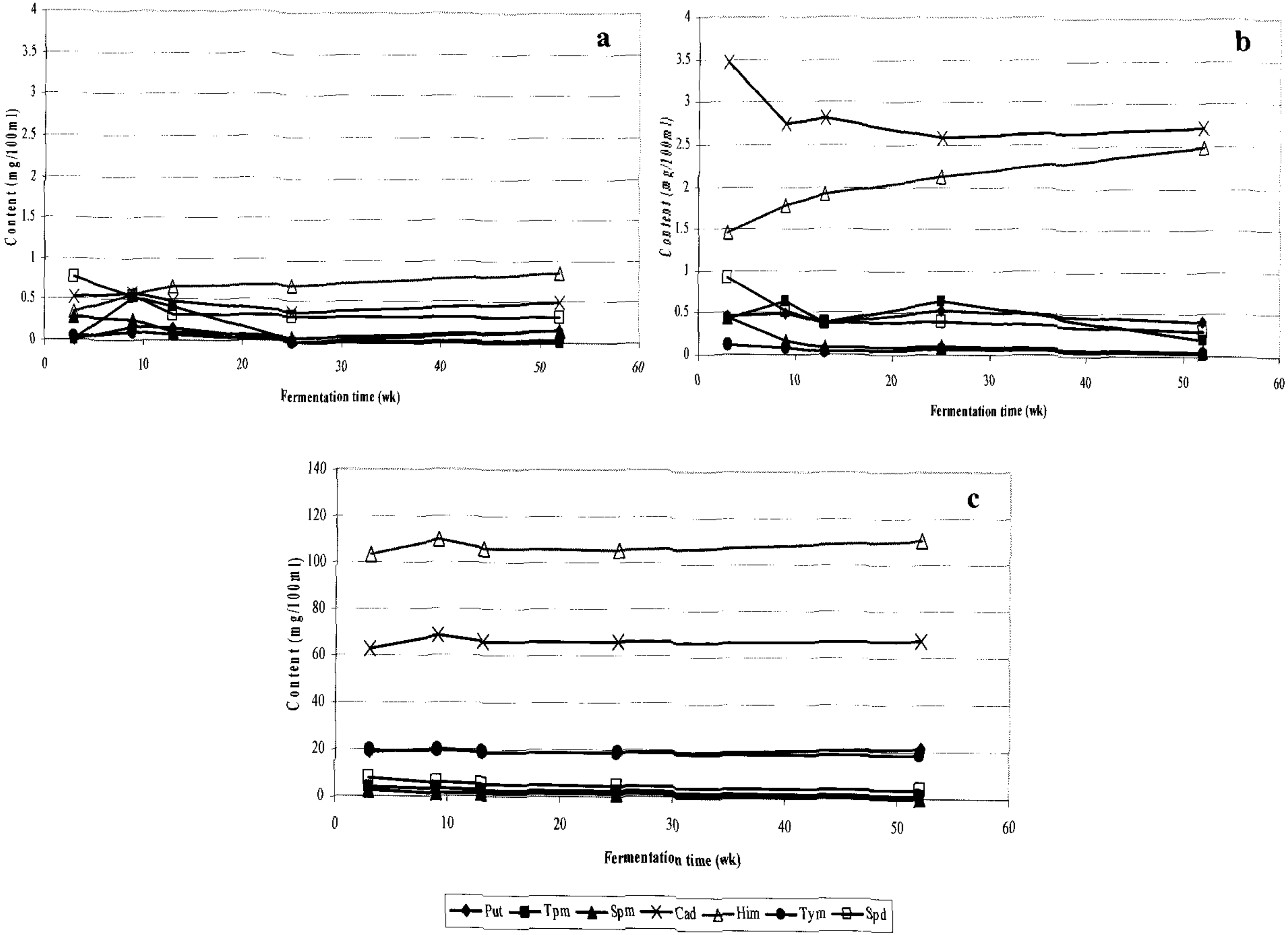
Figure 1. Biogenic amine contents of fish sauce prepared from fresh Indian anchovy (a) and fish stored at 35 °C for 8 (b) and 16 h (c) and fermented at room temperature (28-30 °C). Put, putrescine; Tpm, tryptamine; Spm, spermine; Cad, cadaverine; Him, histamine; Tym, tyramine; Spd, spermidine.

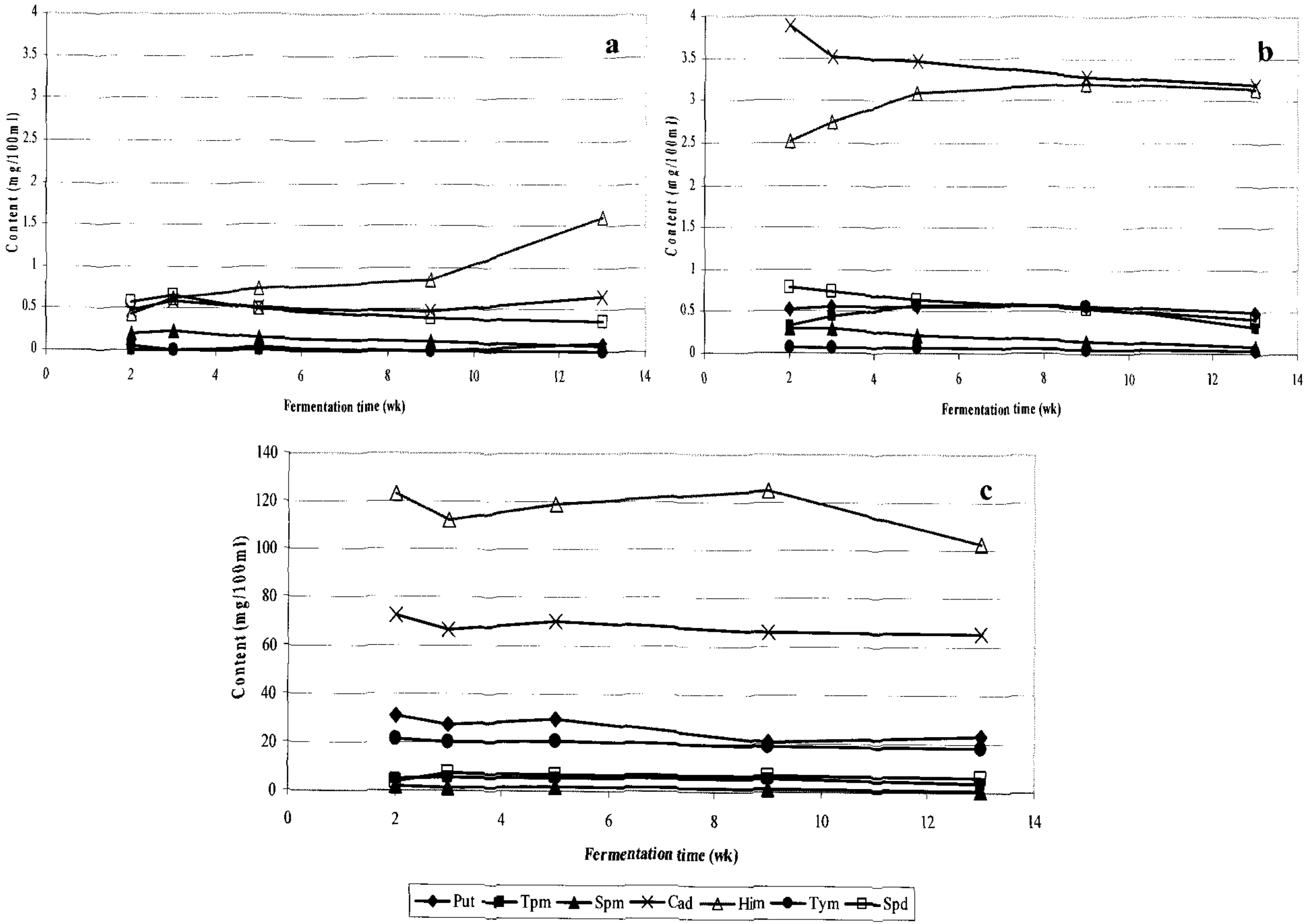
Figure 2. Biogenic amine contents of fish sauce prepared from fresh Indian anchovy (a) and fish stored at 35 °C for 8 (b) and 16 h (c) and fermented at 40 °C. Abbreviations are the same as indicated in Figure 1.

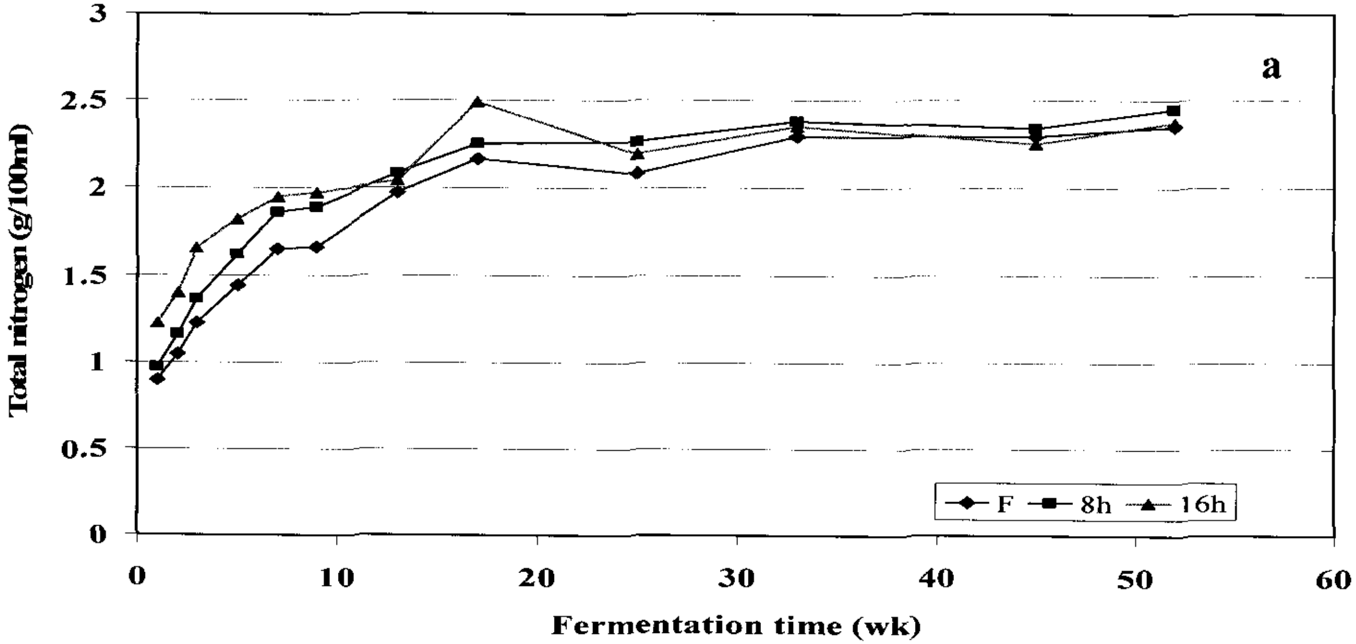
Figure 3. Changes of α-amino contents of fish sauce fermented at room temperature (a) and at 40 °C (b). F is denoted for fresh anchovy, 8h and 16h are anchovy stored at 35 °C for 8 and 16 h, respectively.

Figure 4. Changes of total nitrogen contents of fish sauce fermented at room temperature (a) and at 40 °C (b). F, 8h, 16h are the same as indicated in Figure 3.

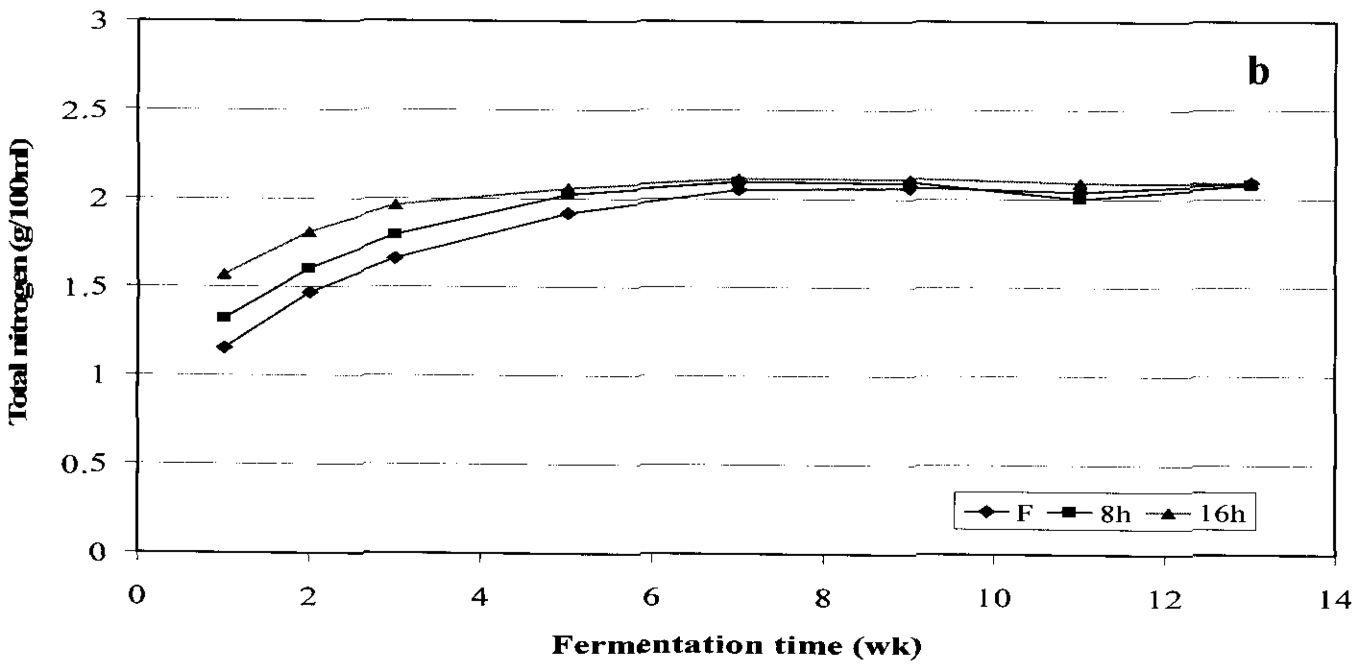
Figure 5. Free amino acid contents of fish sauce fermented at room temperature (a) and at 40 °C (b). F, 8h, 16h are the same as indicated in Figure 3.


a
b
c
Content (mg/100ml)
Fermentation time (wk)
Put
Tpm
Spm
Cad
Him
Tym
Spd

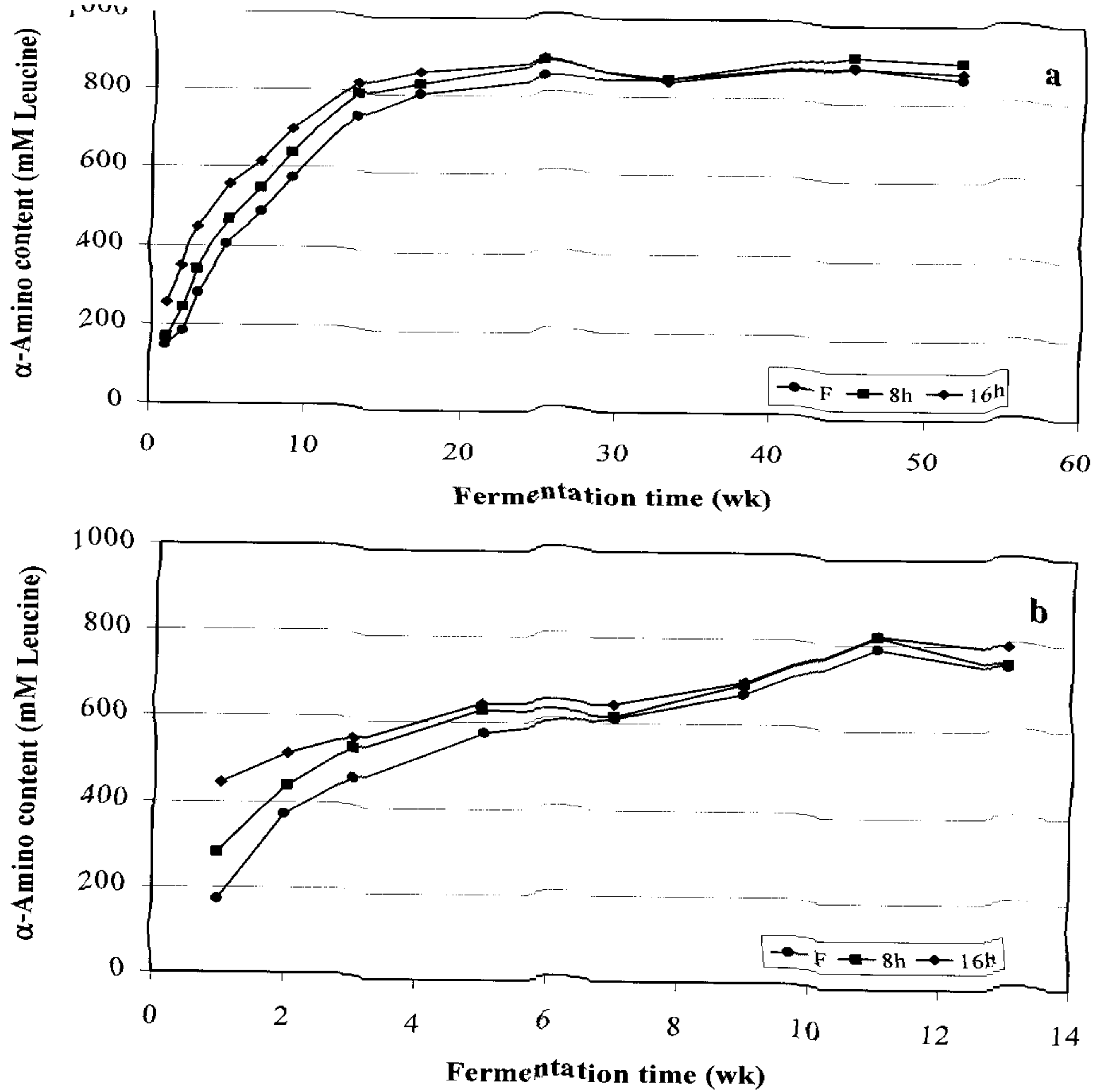

a
b
c
Content (mg/100ml)
Fermentation time (wk)
Put
Tpm
Spm
Cad
Him
Tym
Spd



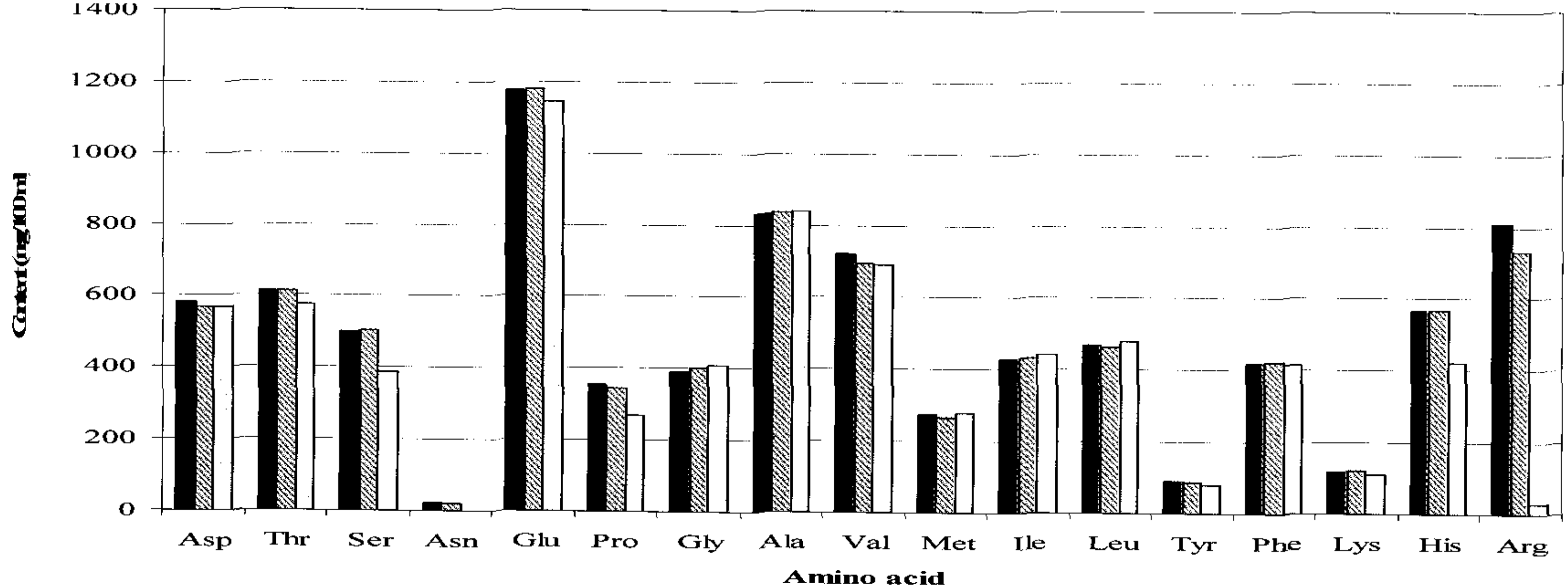
a
Total nitrogen (g/100ml)
Fermentation time (wk)
F
8h
16h



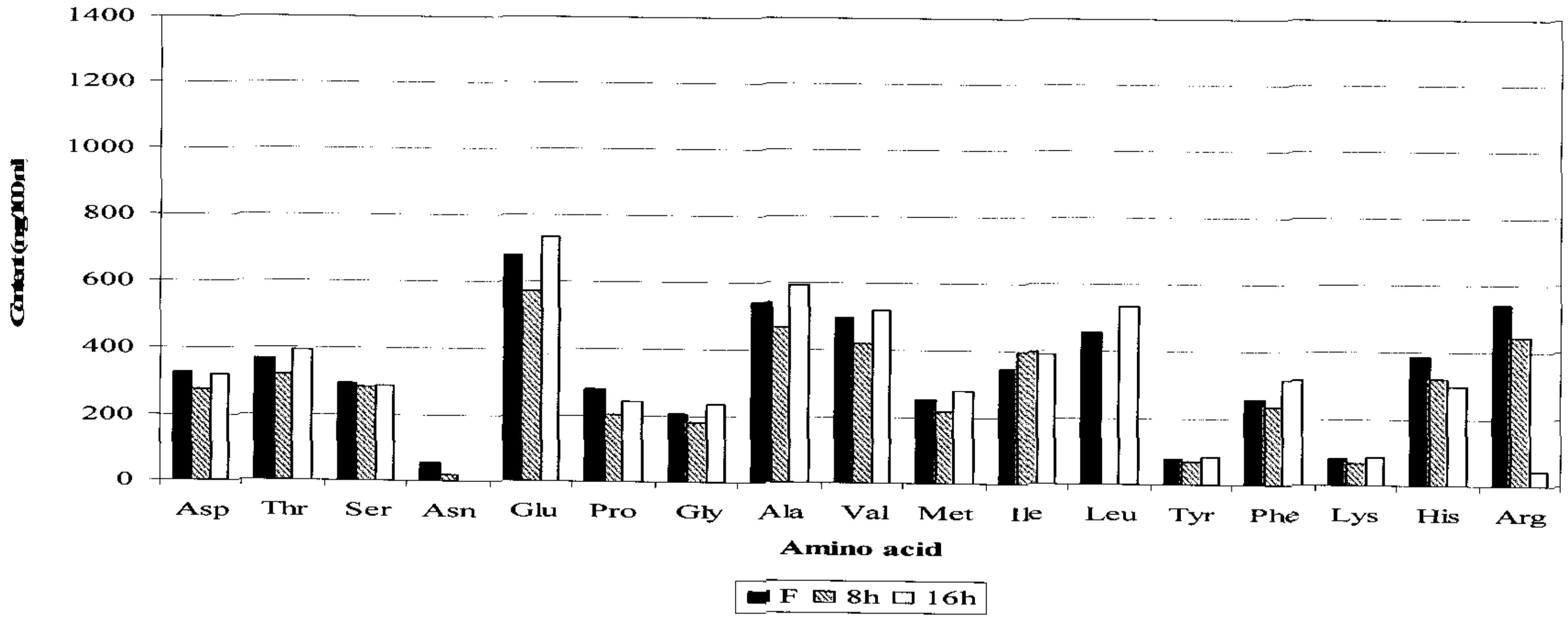
b
Total nitrogen (g/100ml)
Fermentation time (wk)
F
8h
16h



α-Amino content (mM Leucine)
Fermentation time (wk)
a
F
8h
16h
α-Amino content (mM Leucine)
Fermentation time (wk)
b
F
8h
16h



Content (mg/100ml)
1400
1200
1000
800
600
400
200
0
Asp Thr Ser Asn Glu Pro Gly Ala Val Met Ile Leu Tyr Phe Lys His Arg
Amino acid



Content (mg/100ml)
1400
1200
1000
800
600
400
200
0
Asp Thr Ser Asn Glu Pro Gly Ala Val Met Ile Leu Tyr Phe Lys His Arg
Amino acid
F 8h 16h